PB87-166351

Comparative Characterization of Organic Emissions from Diesel Particles Coke Oven Mains, Roofing Tar Vapors and Cigarette Smoke Condensate

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1986

U.S. Department of Commerce
National Technical Information Service

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/J-86/270	2.	3. RE PB87-166351
4. TITLE AND SUBTITLE (Journal Version) Comparative Characterization of Organic Emissions from Diesel Particles, Coke Oven Mains, Roofing Tar Vapors and Cigarette Smoke Condensate		5. REPORT DATE 1986 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R. Williams*, C. Sparacino,@ B. Petersen& and J. Bumgarner, R.H. Jungers and J. Lewtas$		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS EHRT, RTP, NC* RTI, RTP, NC@ BCL, Columbus, OH& USEPA, OHR, RTP, NC$		10. PROGRAM ELEMENT NO. A9RA1B 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/11

15. SUPPLEMENTARY NOTES
Intern. J. Environ. Anal. Chem., 1986, Vol. 26, pp. 27-49

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 24
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA/600/J-86/270
JOURNAL ARTICLE

Intern. J. Environ. Anal. Chem., 1986, Vol. 26, pp. 27-49
0306-7319/86/2601-0027 $18.50/0
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Comparative Characterization of Organic Emissions from Diesel Particles, Coke Oven Mains, Roofing Tar Vapors and Cigarette Smoke Condensate

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(*Received December 16, 1985*)

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This paper reports the characterization of the extractable organics from diesel particulate emissions compared to other complex organics which have been reported to increase the risk of human lung cancer. Class fractions of diesel, cigarette smoke condensate, roofing tar, and coke oven extracts were obtained using liquid/liquid partitioning and silica gel chromatography. Capillary GC/MS was used to identify compounds in each extract fraction. This manuscript reports the mass distribution after fractionation of each extract, all identified fraction components and quantification of selected mutagenic and carcinogenic compounds.

KEY WORDS: Diesel exhaust, coke oven mains, roofing tar, cigarette smoke condensate, class fractionation, mutagenic activity.

INTRODUCTION

Concerns over human health risks caused by inhalation of diesel exhaust have been raised due to studies revealing mutagenic and carcinogenic activity of diesel particle extracts in laboratory experiments.[1-6] Because of this concern, an assessment of the potential increase in human lung cancer was undertaken by the U.S. Environmental Protection Agency.[7] The chemical and toxicological properties of diesel emissions were assessed via comparison with other complex emissions.[8] Cigarette Smoke Condensate (CSC), Coke Oven Mains (CO), and Roofing Tar (RT) were selected as comparative emissions based upon evidence that these emissions result in excess lung cancer risk in studies of humans exposed to relatively high doses of these emissions.[8] The mutagenicity and carcinogenicity of these emissions has been reported elsewhere.[9-14]

This paper reports the chemical characterization of the class fractions from each of these organic emissions extracts that were amenable to direct GC/MS analysis. Emphasis was placed on characterization of the most mutagenic fractions. The mutagenicity data is reported in depth elsewhere.[15] Levels of specific polynuclear aromatic hydrocarbons (PNAs) and nitrosubstituted PNAs which have been reported to be responsible for a high degree of mutagenic activity in some environmental mixtures[16-18] are reported.

EXPERIMENTAL

Glassware and chemicals

All glassware used in the chemical fractionation of the samples was

washed with a soapy solution followed by a deionized water rinse. Glassware was then rinsed with dichloromethane (DCM) followed by heating at 400°C for 4 hr. Prior to actual use, glassware was rinsed with the solvent to be used. All solvents were distilled in glass and were spectrometry grade (Burdick and Jackson).

Diesel automotive emissions

The diesel automotive exhaust emissions were obtained from a light-duty diesel passenger car (Oldsmobile 350). To simulate actual driving patterns, the vehicle was operated with No. 2 diesel fuel on a chassis dynamometer, using the highway fuel economy test cycle (HWFET).[19] The HWFET has an average speed of 48 mph (77.3 km/hr) for 12.75 minutes over 10.24 mi (16.49 km). Exhaust particulates from the diesel engine were collected on 20×20 in. Pallflex T60-A20 Teflon coated glass fiber filters at a flow rate of 100 cfm using the dilution tunnel sampling technique.[20]

Cigarette smoke condensate

As described elsewhere, the cigarette smoke condensate (CSC) was generated and collected utilizing a cigarette smoking machine which condenses the smoke from 2000 cigarettes/hr in acetone cold traps.[8] The cigarettes chosen to make the CSC were the Kentucky Reference 2R1, a remake of the widely studied 1R1. The cigarettes were 85 mm (king size), non-filtered, typical of U.S. cigarettes consumed from 1962–1966, with a tar content of 36.6 mg per cigarette. The CSC was removed by rinsing the trapping system with a minimal amount of acetone.

Coke oven emissions

The coke oven mains were collected from a separator between the gas collector main and the primary coolers within the coke oven battery located at Republic Steel in Gadsden, Alabama, approximately 97 km north-east of Birmingham.[7] The coke oven main sample represented a sample of the vapors and other by-products normally recovered in the by-product gas collector during the "charging" operation of the coking cycle.

Roofing tar emissions

Roofing tar emissions were generated by heating coal-tar pitch to ca. 182°–193°C for 8 hr in a conventional tar pot with an external burner to maintain the tar in liquid form. A 1.83 m stack extension was added to the tar pot to prevent the external burner emissions from entering the sampling hood mounted over the pot. A Teflon-coated aluminum pipe led from the hood to a small baghouse where special non-reactive Teflon filter bags were fitted to collect the particulate emissions.[8] The tar utilized in this study was derived from coal and has been incorrectly reported both as an asphalt[9] and pine pitch[2] tar.

Extraction

The particulate emissions collected from the roofing tar pot and diesel vehicle were Soxhlet-extracted with dichloromethane (DCM) using reported procedures.[8] The coke oven mains were totally DCM soluble and were not Soxhlet-extracted. The CSC was removed from the condenser by rinsing with a minimal amount of acetone and the acetone was removed from the sample by rotary evaporation. The solvents used to extract or dissolve the organics from each sample were removed by evaporation under nitrogen and the samples stored at or below −80°C in the dark.

Chemical class fractionation by liquid/liquid partitioning

Approximate 1 gram quantities from each extract (after dissolution in DCM and 0.5μ Teflon filtering) were fractionated by a liquid/liquid partitioning scheme previously described by Kolber *et al.*[21] This partition scheme, as summarized in Figure 1a, fractionates the sample into organic acids (ACID), organic bases (BASE), cyclohexane insolubles (CI), polar neutrals (PN), non-polar neutrals (NPN) and polynuclear aromatic hydrocarbons (PNA).

After partitioning, the solvent volume in each fraction was reduced to ca. 5 ml using rotary evaporation (reduced pressure at 35°C). Aliquots of each fraction were transferred to tared 1 dram glass vials and taken to dryness using a stream of dry nitrogen. To ensure total solvent removal, each fraction was dried until a repetitive mass value was obtained ($\pm$0.1 mg).

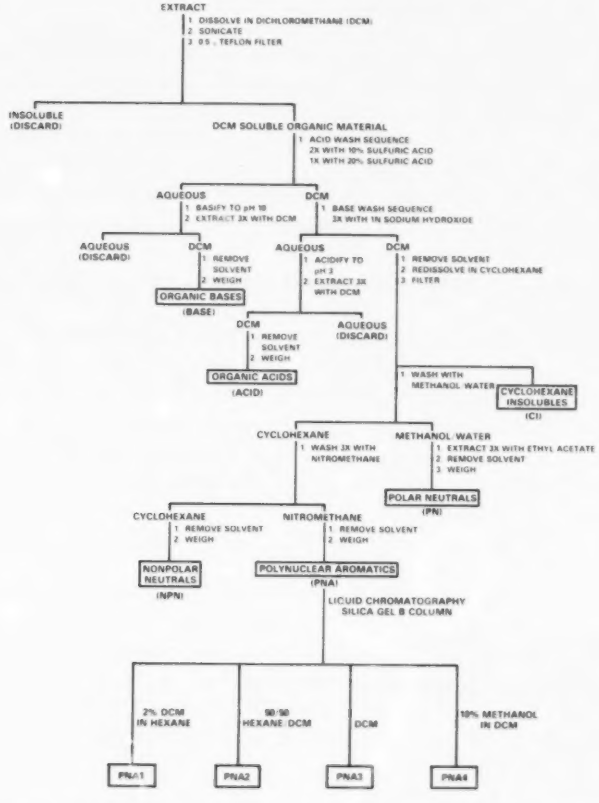

FIGURE 1a Liquid/liquid fractionation and low pressure liquid chromatography partitioning scheme used upon emission extract samples.

A separate fractionation of milligram quantities of each extract utilizing silica gel column chromatography was used to prepare samples for quantification of nitroaromatics as described later. The mass distribution and recovery for both fractionation procedures are reported, however the characterization and mutagenicity of only the liquid/liquid partitioned samples were examined.

Subfractionation of PNAs

The nitromethane fraction from the liquid/liquid partitioning, which contained PNAs, was further fractionated as shown in Figure 1a using preparative scale liquid chromatography. A low pressure Lobar® silica gel B column (E. Merck) was used in conjunction with a Perkin Elmer series 1 pump operating at 29.9 ml/min with a pulse dampener (FMI) and a UV-254 nm detector (Gilson) in line.

Milligram quantities were introduced to the column by means of a 3-way slide injection valve (Rainin). All contact surfaces in the system were of a non-contaminating nature (Teflon®, stainless steel, glass).

The PNA hydrocarbons were eluted from the column using 2% DCM in hexane. This fraction was designated as PNA 1. Standard solutions of phenanthrene and coronene were eluted in this fraction within 24 minutes with a ten minute period between their respective elution peaks. More polar material (e.g., functionalized PNAs) were eluted in subsequent 10 minute periods using progressively more polar solvent compositions. Fraction PNA 2 was eluted with 50% DCM in hexane, PNA 3 with 100% DCM and PNA 4 with 10% methanol in DCM.

The PNA fractions from the diesel and roofing tar samples were subfractionated into PNA 1–4 using this method. The CSC and coke oven PNA fractions were also subfractionated except that the PNA 2 (50% DCM in hexane) was eliminated in the elution program. PNA 3 for these fractions thus contained material normally eluting in subfractions PNA 2–3. Solvent was removed from each fraction as previously described.

Gas chromatography/mass spectroscopy

Aliquots of the emission extracts liquid/liquid partition fractions, including their PNA 1 fractions were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) in the attempt to tentatively identify compound types in each sample. Mass spectral data (total ion current profiles and individual mass spectra) was obtained using an LKB 2091 GC/MS operated in the positive ion electron impact mode at 70 ev.[21] Fractions were analyzed using either a 24 m Support Coated Open Tubular (SCOT) CP-SIL 5 or a 25 m SCOT SE-52 DM 0.25 mm i.d. column using linear temperature

programming. Mass spectral scans were taken every 2 seconds with a range of 50–492 amu. Identification of mass spectra was accomplished through comparison of spectra to the Aldermaston 8 Peak Index of Mass Spectra.

Silica gel fractionation/preparation for nitroaromatic analysis

Extract aliquots in the range of 50 to 100 mg were dissolved in hexane and placed upon activated beds of silica gel (Woelm Pharma, 70–150 mesh), slurry packed and capped with a layer of anhydrous sodium sulfate in a 25 × 200 mm glass column. The mass of silica gel used for each fractionation was adjusted to give a 200:1 ratio to the extract mass. Using an attached solvent reservoir, four sequential solvent eluants were passed through the column at a flow rate of 2 ml/min. The elution volume of each solvent was fixed to give a 15:1 ratio to the silica mass (ml/g).

Hexane was utilized as the initial elution solvent to remove aliphatic hydrocarbons from the column. This was followed by a 1:1 mixture of hexane/benzene which eluted aromatics and mononitrated aromatic species. More polar solvents, dichloromethane followed by methanol, were then used to remove moderate and highly polar neutral species respectively (Figure 1b). This later fraction consisted mainly of oxygenated compounds. Each fraction was concentrated to ca. 5 ml using rotary evaporation. Aliquots were removed and dried to a constant weight to determine mass recovery.

Quantification of aromatic species

Nitrosubstituted PNAs were quantified in the emission extract fractions by capillary GC/negative chemical ionization mass spectrometry (NCI/MS).[16] NCI/MS is superior to positive ion detection modes for the analysis of nitrosubstituted PNAs due to the electronegativity of the nitro moiety. The detection limit for 1-nitropyrene by this technique is 50 pg injected on-column. The GC/MS system was a Finnigan 4000 GC/MS interfaced to a INCOS 2300 computer. The gas chromatographic column was a 30 m × 0.30 mm i.d. fused silica capillary. Methane was used as the carrier gas at a linear velocity of 30 cm/sec at 100°C. Samples were injected on-column and eluted using a linear temperature program 40°–320°C at 10°C/min.

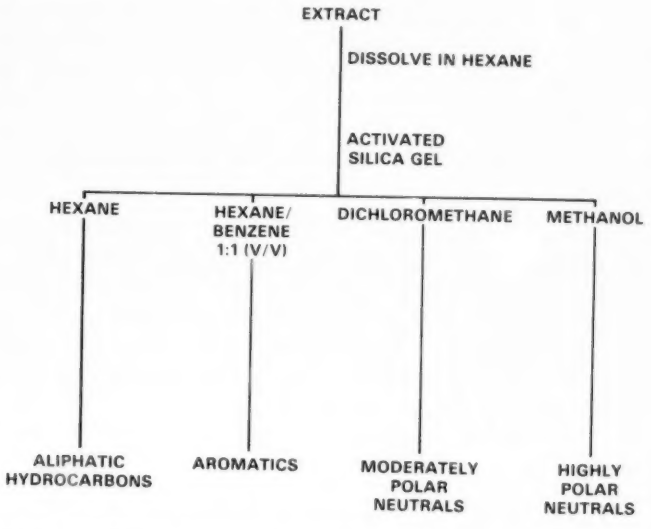

FIGURE 1b Silica gel fractionation of emission extracts for nitroaromatics analysis.

Nitroaromatics were quantified by comparing their GC/MS response to reference standards. Procedures used to quantify benzo(a)pyrene in the four unfractionated emissions (cyclohexane extraction, thin layer chromatography of extract followed by fluorescence scanning of the chromatography plates with results compared to reference standards) have previously been reported.[22,23]

RESULTS AND DISCUSSION

Distribution of mass and recovery of material from the two fractionation procedures are listed in Table I. These data demonstrate that both procedures resulted in acceptable mass recovery (83–94%) for the diesel, coke oven and roofing tar extracts. A low mass recovery for the CSC sample was obtained when it was partitioned with solvent (47.6%). The reason for this low recovery may be due in part to the incomplete extraction of organic acids. Previous reports of CSC extracts indicate higher organic acid content.[24,25]

TABLE I

Mass distribution and recovery of emission extracts after fractionation

Fraction method	Diesel		CSC		Coke oven		Roofing tar	
a. Liquid Partitioning fractions[a]								
Acid	25.3	(3.6)	4.4	(1.0)	2.7	(0.3)	20.2	(2.3)
Base	7.3	(1.0)	53.0	(12.2)	30.8	(3.9)	25.8	(2.9)
NPN	523.3	(75.1)	2.8	(0.6)	108.5	(13.9)	361.9	(40.4)
PNA[b]	31.0	—	2.6	—	139.3	—	298.9	—
PNA1	—	(1.1)	—	(0.01)	—	(9.0)	—	(31.8)
PNA2	—	(1.4)	—	ND[c]	—	ND[c]	—	(1.2)
PNA3	—	(0.7)	—	(~0.07)[c]	—	(7.4)[c]	—	(~0.02)
PNA4	—	(1.3)	—	(0.52)	—	(1.4)	—	(0.3)
PN	53.7	(7.7)	50.4	(11.6)	50.7	(6.5)	117.1	(13.1)
CI	56.3	(8.1)	321.8	(74.0)	451.1	(57.6)	72.0	(8.0)
Mass fractionated (mg)	803.0		913.8		936.8		1071.2	
Mass recovered (mg)	696.9		435.0		783.1		895.5	
% recovery	86.8		47.6		83.6		83.6	
b. Silica Gel fractions[a]								
Aliphatics	64.4	(70.5)	1.6	(3.6)	1.6	(3.5	22.6	(21.8)
Aromatics	3.8	(4.2)	2.4	(5.1)	24.8	(54.5)	61.3	(59.2)
Moderately polar neutrals	2.1	(2.3)	8.8	(18.8)	14.4	(31.7)	6.5	(6.3)
Highly polar neutrals	21.0	(23.0)	33.8	(72.5)	4.7	(10.3)	13.2	(12.7)
Mass fractionated (mg)	96.7		54.0		52.0		117.3	
Mass recovered (mg)	91.3		46.6		45.5		103.6	
% recovery	94.5		86.2		88.3		88.3	

[a]Values in ( ) represents the % fraction contribution to total recovered mass.

[b]A portion of the PNA liquid/liquid partition fraction was further fractionated using silica gel chromatography. Values in ( ) represent the calculated % each subfraction would have contributed to the total recovered mass if the entire PNA partition fraction had been subfractionated.

[c]ND = Not determined, in these samples the solvent used to elute the PNA2 fraction was omitted. Therefore, any mass that would have eluted in PNA2 would elute in PNA3.

Contribution of acids and bases were low for emission extracts fractionated by liquid partitioning (Table Ia). A majority of the mass was located in the four neutral fractions (PN, NPN, CI and PNA). The highest percentage contribution of acid mass was found in the diesel extract (3.6%). This extract also had the lowest percentage of organic bases (1.0%). As reported here and observed by others[24,25], the base fraction of the CSC extract contributes a moderate percentage to the total mass recovery (12.2%).

Within the neutral fractions, the four extracts were found to have a distribution of mass characteristic of the emission. The diesel neutral fraction was dominated by aliphatics as seen in the NPNs of the liquid partition (75.1% total mass) and in the aliphatic fraction of the silica gel separation (70.5%). Other studies[18,26] of diesel particle extracts have reported high percentages (57–71%) of C_{14}–C_{35} aliphatic hydrocarbons to be present, which is thought to result from the incomplete combustion of diesel fuel.[27] Mass from polar neutral type species, as seen in the PN and CI fractions from liquid partitioning (sum of 15.8%) and the highly polar neutral fraction from the silica gel fractionation (23.0% of the total) were found in significant quantities in the diesel sample. PNAs from this diesel sample contributed less than 5% of the total mass fractionated in the liquid partitioned sample (4.5% total mass) as well as in the aromatic fraction of the silica gel fractionation method (4.2%). Subfractionation of the liquid partitioned PNA diesel fraction suggests that only 1.1% of the total mass was PNA hydrocarbons (PNA 1).

A majority of the recovered mass from the CSC separation appears to be moderately and highly polar neutral compounds. In the silica gel fractionation, 91.3% of the recovered mass was in the polar fractions while in the liquid partitioning fractionation 85.6% of the mass was found in the CI and PNs. Aliphatic hydrocarbons and PNAs were recovered in small quantities ($<5.1\%$ of mass) with both methods (Table I). Others have reported relatively low masses of PNAs and aliphatics in other CSC samples together with higher masses of polar neutral species.[24,25]

Mass recovery from the coke oven fractionations indicate that the liquid partition and column chromatography results differ significantly for this extract. This may be due to the large portion of cyclohexane insoluble (CI) material (57.6%) which could not be partitioned into the PNA or PN fraction by the liquid/liquid

method. This may therefore account for the lower PNA content (17.8%) compared to the 54.5% observed by silica gel fractionation.

The roofing tar sample was found to be dominated by the aliphatic, and aromatic fractions from both methods. Differences were observed between mass percent recovery of these fractions in the two schemes such as a 21.8% recovery of aliphatics by the silica gel separation as compared to 40.4% NPN by the liquid partitioning. The silica gel fractionation of the roofing tar samples resulted in 59.2% of the mass in the aromatic fraction while only 33.3% of the recovered mass from liquid partitioning was found in the PNAs (PNA 1–4). Subfractionation of the PNAs of this sample revealed a large quantity (31.8%) of parent or simple substituted PNA species found in the PNA 1 subfraction (Table Ia). Both separation schemes indicated the presence of moderate mass quantities of polar and highly polar species.

GC/MS data revealed that polycyclic organic species were present in all of the extract samples and many of these species were found in more than one fraction. The majority of the acids identified by GC/MS were from the CSC extract fraction. As seen in Table II, vegetative combustion products containing hydroxylated aromatic species (phenols, catechols, etc.) were identified in the CSC extract. A number of PNA and alkylsubstituted PNA derivaties of carboxylic acid found in the most polar fractions from diesel emission extracts[18] were not detected in either the acid or polar neutral fractions by the GC/MS method reported here.

Very few components were identified in the base fraction from the four samples (Table III). Nitrogen containing heterocyclics such as carbazole and methylbenzoquinoline were detected in the coke oven and roofing tar samples. The CSC revealed only the presence of nicotine and one other nitrogen containing structure ($C_{13}H_9N$), although a number of aromatic arenes, including nitrosoamines have been reported in tobacco smoke.[28] Nitrogen containing heterocyclics such as acridine have been reported in diesel extracts.[18]

Since the compounds identified in the PN and CI fractions were similar and in some cases identical, they are reported in Table IV. The coke oven and roofing tar samples were found to contain similar parent PNAs (e.g. naphthalene, fluoranthene and perylene) which appeared in these polar fractions. Nitrogen and sulfur heterocycles (e.g. carbazole and dibenzothiophene), oxygenated PNAs

TABLE II

Identification of compounds in the organic acid fractions by GC/MS

Compound	Emission extracts			
	Diesel vehicle	CSC	Coke oven	Roofing tar
Phenol		+		
Cresols		+		
o-Methoxyphenol		+		
Xylenols		+		
Ethylphenol		+		
i-Propylphenol		+		
C-3 phenol		+		
C-4 phenol		+		
Methylbenzothiophene		+		
i-eugenol		+		
C-2 benzothiophene		+		
C-4 catechol		+		
Naphthol		+	+	+
C-3 benzothiophene		+		
Hydroxy-*i*-carvomenthol		+		
C-2 naphthiridine		+		
i-Menthone		T		
Methylbenzofuran		T		
Methoxythioanisole		T		
Methallyl phenol		T		
Eugenol		T		
Dimethyldihydroindene		T		
Dibenzofuran		T		+
2-ethylbiphenyl		T		
Acenaphthene				+
Fluorene				T
Methoxynaphthalene				+
$C_{14}H_{10}$				+
$C_{16}H_{10}$			+	+
$C_{17}H_{12}$			+	T
Phenylphenol				
$C_{12}H_8$			+	
Tetracosanoic acid			T	
Saturated HC	+			
Unsaturated HC	+			
1, 1, 3-Me_3-3-phenylindan	+			
2, 6-di-*t*-butyl-4-ethylphenol	T			
Methylphenanthrene	+			
$C_{13}H_{17}O$	+			

+ = identified by matches with Aldermaston Eight Peak Index.

T = tentatively identified based upon GC retention data and/or MS fragmentation due to an inconclusive match through use of the Eight Peak Index.

TABLE III

Identification of compounds in the organic base fractions by GC/MS

Compound	Emission Extracts			
	Diesel vehicle	CSC	Coke oven	Roofing tar
Isobutylisobutryramide	(uninterpretable)[a]		T	
Carbazole			T	T
Borazophenanthrene			+	T
$C_{13}H_9N$			T	
Naphthoquinaldine			T	
Methylbenzoquinoline			T	+
Dibenzoheptafulvene			T	+
Cyanoanthracene			+	
Nicotine		+		
$C_{10}H_8N_2$		+		
Perylene		+		
Saturated HC				T
$C_{10}H_{14}$				T
Acridine				+
$C_{13}H_{23}N$				+
$C_{16}H_{12}$				+

+ = identifed by matches with Aldermaston Eight Peak Index.
T = tentatively identified based upon GC retention data and/or MS fragmentation due to an inconclusive match through use of the Eight Peak Index.
[a]GC/MS techniques employed failed to resolve components sufficiently.

including quinones (e.g. anthroquinone) and hydroxylated PNAs (e.g. hydroxyfluorene) were found in the diesel, coke oven and roofing tar samples in the PN and CI fractions.

The NPN fractions (Table V) were found to contain aliphatic hydrocarbons C_{10}–C_{24} in addition to PNAs and a number of substituted PNAs. The diesel NPN fraction was found to contain large numbers of saturated and unsaturated hydrocarbons all of which were not separately identified. While aliphatic hydrocarbons were identified in the NPN fractions of the CSC and the diesel sample none were positively detected for the roofing tar and coke oven extracts. In fact, for these two extracts, aliphatic hydrocarbons were not found in any of the fractions.

As seen in Table VI substituted and unsubstituted PNA species of up to 5 rings were detected in various PNA 1 fractions. A number of

TABLE IV

Identification of compounds in the cyclohexane insolubles and polar neutral fractions by GC/MS

Compound	Emission extracts			
	Diesel vehicle	CSC	Coke oven	Roofing tar
Naphthalene			+	+
Methylnaphthalene			+	+
Acenaphthene			+	+
Ethylnaphthalene				+
Chloro-*i*-propyltoluene				+
Diethylchlorobenzene				+
Dimethylnaphthalene			+	+
Dibenzofuran			+	+
Fluorene			+	+
Hydroxyfluorene				+
Chloromethylbenzofuran				T
Phenanthrene	T			
Fluorenone	+			
Methylphenanthrene	+		+	+
Carbazole			+	+
Methylcarbazole				+
Dibenzothiophene			+	+
Methyldibenzothiophene	T			
Dibenzoheptafulvene			+	+
Fluoranthene			+	+
Pyrene			+	+
Dihydrobenzcarbazole				T
Fluorene carbonitrile			+	T
Methylpyrene			+	T
Phenylphenol	+			
Methylfluorenone	+			
Benzophenanthrene				+
Diphenylacetylene	T			
Diphenyldiacetylene	T		+	
Perylene				T
Acenaphthylene			+	
Methylacenaphthalene			+	
Benzocinnoline	+			
Methylbenzocinnoline	T			
Anthraquinone	+			
Hydroxyisocarvomenthol		+	T	
Diphenylethylene			+	

TABLE IV (continued)

Compound	Emission extracts			
	Diesel vehicle	CSC	Coke oven	Roofing tar
Methylenephenanthrene			+	
Benzalacetophenone	T			
Dimethylnaphthothiophene	T			
Naphthothianaphthene			+	
Benzofluoranthene			+	
Dihydroxyanthraquinone			T	
Methylbenzanthrylene			+	
Nicotine		+		

+ = identified by matches with Aldermaston Eight Peak Index.
T = tentatively identified based upon GC retention data and/or MS fragmentation due to an inconclusive match through use of the Eight Peak Index.

TABLE V

Identification of compounds in the nonpolar neutral fractions by GC/MS

Compound	Emission extracts			
	Diesel vehicle	CSC	Coke oven	Roofing tar
Acenaphthene			+	+
Dibenzofuran			+	+
Fluorene			+	+
$C_{13}H_{12}$				+
$C_{13}H_{10}O$				+
Hydroxyfluorene			+	+
Dihydroanthracene				+
Dibenzothiophene			+	+
$C_{14}H_{10}$	+			+
Methylphenanthrene			+	+
Methylenephenanthrene			+	+
Dibenzoheptafulvene			+	+
Diphenyl acetylene				+
$C_{16}H_{10}$				+
Unsaturated HC	×	+		
Saturated HC	×	+		
n-Dodecane		+		

TABLE V (continued)

Compound	Emission extracts			
	Diesel vehicle	CSC	Coke oven	Roofing tar
Pentadecene		+		
n-octadecane	+	+		
n-tetracosane		+		
Octadecyl fluoride		+		
Naphthalene			+	
Methylnaphthalene			+	
Biphenyl			T	
Dimethylnaphthalene			+	
Acenaphthylene			+	
Methylbiphenyl			+	
Methylacenaphthylene			+	
Methylfluorene			+	
Anthracene/phenanthrene			+	
Methyldibenzothiophene			+	
Dimethylphenanthrene			+	
Fluoranthene			+	
Pyrene			+	
Decahydropyrene			T	
Methylpyrene			+	
Bi-indolyl			T	
Terphenyl			+	
Naphtho(2, 1-b)thianaphthene			+	
Chrysene/triphenylene			+	
Methylchrysene			+	
n-Heptadecane	+			
$C_{20}H_{42}$	+			
$C_{19}H_{40}$	+			
$C_{21}H_{44}$	+			
$C_{22}H_{46}$	+			
$C_{23}H_{48}$	+			

+ = identified by matches with Aldermaston Eight Peak Index.
T = tentatively identified based upon GC retention data and/or MS fragmentation due to an inconclusive match through the use of the Eight Peak Index.
× = while definite hydrocarbon fragmentation patterns were present no indentification could be made.

TABLE VI

Identification of compounds in the PNA 1 fractions by GC/MS

Compound	Emission extracts			
	Diesel vehicle	CSC[a]	Coke oven	Roofing tar
Phenanthrene/anthracene	+	+	+	
Diphenylacetylene	T			
Methyldibenzothiophene	+			
Methylphenanthrene	+	+	+	+
Dimethylnaphthothiophene	+			
Methylanthracene		+		
Methylbenzodithiophene	T			
Methoxyanthracene	T			
C-2 phenanthrene	+			+
Fluoranthene	+	+	+	
Pyrene	+	+		+
C-3 phenanthrene	+			
Methylpyrene	+	+	+	+
Dimethylpyrene	+			
p-Terphenyl		+		
Benzo(a)fluorene		+		
Benzo(ghi)fluoranthene/benzo(a)phenanthrene		+		
Benz(a)anthracene		+		
Methylchrysene		+		
Benzo(b/j/k)fluoranthenes		+		
Benzo(a)pyrene[b]	+	+	+	+
Benzanthrone	T			
Chrysene	+	+	+	
Fluorenone	+			
Benzofluoranthene	+			
Methylfluorenone	+			
Anthraquinone	+			
Phenylphenol	T			
Acenaphthene				+
Dibenzofuran			+	+
$C_{13}H_{10}$				+
$C_{13}H_{10}O$				+
Dihydroanthracene				+
Methylenephenanthrene			+	+
Dibenzoheptafulvene			+	T
$C_{17}H_{12}$				+
Naphthalene			+	+
Methylnaphthalene			+	+
Methylbiphenyl				+
Hydroxyfluorene			+	+

TABLE VI (continued)

Compound	Emission extracts			
	Diesel vehicle	CSC	Coke oven	Roofing tar
Methylfluorene			+	+
Diphenyldiacetylene				+
Benzofluorene			+	+
Naphthothianaphthene			+	+
Methylbenzoanthracene				+
Methylacephenanthrene				+
Perylene	+		T	+
Perylene isomer			+	+
Dimethyl naphthalene			+	
Acenaphthylene			+	
Methylacenaphthylene			+	
Fluorene			+	
Dibenzothiophene			+	
Benzophenanthrene			+	
$C_{19}H_{14}$			+	
Dihydroxyanthraquinone			+	

+ = identified by matches with Aldermaston Fight Peak Index.

T = tentatively identified based upon GC retention data and/or MS fragmentation due to an inconclusive match through use of the Eight Peak Index.

[a]Compounds were not identified in this PNA 1 fraction and are listed for comparison purposes only, species were detected in an ORNL analysis of this Kentucky 2R1 CSC using different methods (Reference 31).

[b]BaP was identified in a separate analysis (Reference 23) of the emission extracts but was not positively identified in the extracts PNA 1 fraction.

the PNAs were also identified in the CI and PN fractions. Alkylated, hydroxylated, and oxygenated PNA species were also present in the PNA 1 fraction.

Quantitative analysis for nitroaromatics was performed on the aromatic and moderately polar silica gel fractions. The total concentration by weight in parts per million (ppm) in each emission extract is reported in Table VII. The diesel particulate extract was the only solution which contained detectable levels of mononitrated aromatics, of which, 1-nitropyrene was found in the highest concentration (107 ppm). None of the extremely mutagenic dinitropyrenes (1,3–; 1,6–; 1,8–) were found in any of these four extracts,

TABLE VII

Concentration (ppm) of selected nitroaromatics and benzo(a)pyrene in emission extracts

Compound	Diesel	Emission extracts: Coke oven mains	Roofing tar	CSC
1-nitronaphthalene	0.3	ND[a]	ND[a]	ND[a]
Nitronaphthalene/azulene isomer	0.2	ND[a]	ND[a]	ND[a]
Nitronaphthalene/azulene isomer	0.3	ND[a]	ND[a]	ND[a]
Nitrofluorene isomer	0.5	ND[a]	ND[a]	ND[a]
2-nitrofluorene	0.4	ND[a]	ND[a]	ND[a]
Nitrofluorene isomer	1.1	ND[a]	ND[a]	ND[a]
Nitrophenanthrene/anthracene isomer	63	ND[a]	ND[a]	ND[a]
Nitrophenanthrene/anthracene isomer	25	ND[a]	ND[a]	ND[a]
1-nitropyrene	107	ND[a]	ND[a]	ND[a]
Nitromethylpyrene isomer	22	ND[a]	ND[a]	ND[a]
Nitromethylpyrene isomer	1.9	ND[a]	ND[a]	ND[a]
Nitro 228[b]	1.4	ND[a]	ND[a]	ND[a]
Nitro 228	0.2	ND[a]	ND[a]	ND[a]
Nitro 228	1.2	ND[a]	ND[a]	ND[a]
Nitromethyl 228	ND	ND[a]	ND[a]	ND[a]
Nitro 252[c]	1.1	ND[a]	ND[a]	ND[a]
Nitropyrenone isomer[d]	2.1	ND[a]	ND[a]	ND[a]
Nitropyrenone isomer	3.9	ND[a]	ND[a]	ND[a]
1, 3-dinitropyrene	ND	ND[a]	ND[a]	ND[a]
1, 6-dinitropyrene	ND	ND[a]	ND[a]	ND[a]
1, 8-dinitropyrene	ND	ND[a]	ND[a]	ND[a]
BaP	2	1758	889	0.6

[a]Not detected (< 50 pg). Reference 16
[b]PAH isomers of MW 228-benz(a)anthracene, chrysene, benz(c)phenanthrene, etc.
[c]PAH isomers of MW 252-B(e)P, B(a)P, perylene, benzofluoranthenes, etc.
[d]Tentative identification.

however they have been detected in other diesel extract samples.[16] Others have reported diesel extracts to contain 1-nitropyrene in a range of 55–2000 ppm.[16,18]

Quantitative analysis for benzo(a)pyrene (BaP) is also shown in Table VII. The coke oven and roofing tar extracts contained the highest concentration of BaP, 1758 ppm and 889 ppm respectively.

The CSC and diesel sample extracts contained much lower but readily detectable concentrations of BaP (0.6 and 2 ppm respectively).

Mutagenic activity in *Salmonella typhimurium* TA98 is reported in detail elsewhere for the whole emission extracts as well as for their individual liquid partition fractions.[15] It is worthy to note however, that for the unfractionated extracts, only the diesel sample exhibited significant direct acting mutagenicity (2.1 revertants/μgs organics) when assayed by the Ames test without the addition of rat liver microsomes (S9). Induced activity was observed in each extract when they were assayed with activation. As discussed below, induced activity of the extracts was found to be distributed in only a few fractions indicating a concentrations of the mutagenic species during the fractionation procedure. A summary of the distribution of the observed mutagenicity in the presence of metabolic activation for the extracts is shown in Figure 2.

The polar (PN and CI) fractions were found to represent 52.5 and 16.4% of the induced mutagenicity for the diesel sample. Identified nitroaromatic species, which may have been present in these fractions, could only account for a portion of the observed activity.

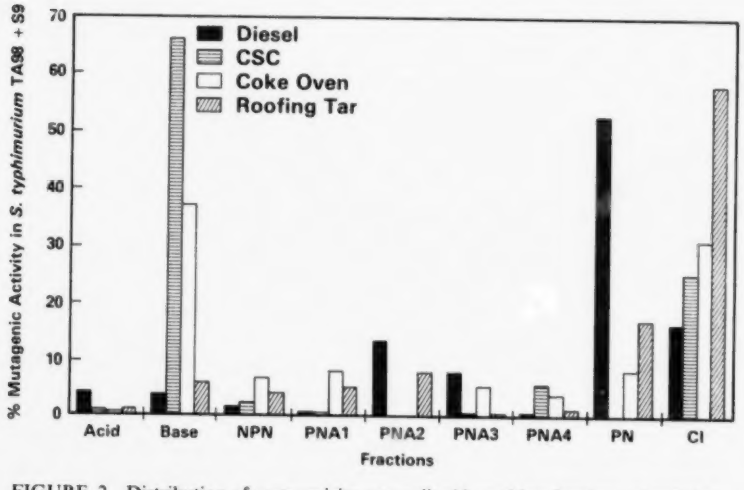

FIGURE 2 Distribution of mutagenicity across liquid partition fractions of emission extracts.

Specifically, 1-nitropyrene and 3-nitrofluoranthene concentrations were calculated to represent only 3.5% of the extract's total direct acting mutagenicity.[29] Furthermore, a maximum of only 11 and 15% of the PN and CI fractions induced activity respectively, could be traced to possible 1-nitropyrene influence (based on extract concentration). Unresolved induced activity in these fractions may be due to oxygenated derivatives of PNAs often found in polar fractions of environmental samples.[17,18,21,30]

Activity in the CSC extract was observed in the base and CI fractions (66 and 25%, respectively). Characterization efforts provided little information about the species present in these fractions although aromatic amines, including nitrosoamines, are known to be present in cigarette smoke.[28] Mutagenicity studies from similarly prepared CSC fractions, have reported that pyrolytic products (e.g. heterocyclic amines) may be responsible for activity within the basic fractions.[25]

Species including amino-PNAs and nitrogen containing heterocycles were present in the coke oven extract where mutagenicity was distributed between the base (37.1%) and CI fractions (31.0%). GM/MS data revealed that oxygen (furans, ketones and quinones) and sulfur heterocycles (thiophenes) were found in the CI fraction. The coke oven extract contained the highest concentration of BaP of the four emissions extracts; 8.4% of the total induced activity could be attributed to this component. This value compares well with the activity of the PNA 1 fraction theoretically containing the compound where 7.9% of the samples activity was exhibited.

Mutagenicity in the roofing tar sample was determined to be primarily in the CI and PN fractions which contained 75.2% of the observed total. Nitrogen, sulfur and oxygenated heterocycles similar to those found in the coke oven were detected. Based upon extract concentration BaP was calculated to contribute only 2.5% of the total activity.

To more fully characterize the mutagens present in these emission extracts, techniques and efforts beyond those employed in this study are necessary. Subfractionation of those class fractions yielding significant levels of mutagenicity followed by further bioassay would help to isolate and document specific components that are major contributors to mutagenicity. Quantification of mutagenic species demand that additional analytical techniques be utilized. Finally,

securing analytical quality standard reference materials for use in quantifying both the chemical concentration and the mutagenicity of the pure compound. This will be necessary to determine the contribution quantified mutagenic species make to overall mutagenicity of emission samples.

Acknowledgements

We would like to thank Jackie Finley for typing and editorial support, M. Nishioka for GC/MS support, K. Brady for technical support, and A. Austin for bioassay support.

Disclaimer

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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